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Volodymyr V. Golotyuk
Associate Professor of Department of oncology, SHEE "Ivano-Frankivsk national medical university", Ivano-Frankivsk, Ukraine

Anatoliy P. Burlaka
Professor of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kiev, Ukraine

Volodymyr V. Golotyuk
Associate Professor of Department of oncology, SHEE "Ivano-Frankivsk national medical university", Ivano-Frankivsk, Ukraine

The Influence of the radiotherapy on the state of the Ceruloplasmin-Transferrin system and free iron combinations' levels in the blood of rectal cancer patients

Volodymyr V. Golotyuk, Anatoliy P. Burlaka

Abstract
The venous blood samples of 35 patients with rectal adenocarcinoma $T_{2-3}N_{0-2}M_0G_2$ have been studied. Patients received preoperative course of distance gamma-ray therapy to the tumour region to a total focal dose of 39 Gy (single focal dose 3 Gy with 13 sessions during 2.5 weeks). A radical surgical intervention was done 4-5 weeks after the last session of radiotherapy. The results of the study show a reduction of ceruloplasmin and transferrin levels in the blood of rectal cancer patients prior to the treatment and a slight growth of transferrin when undergoing the course of radiotherapy. One of the reasons for the decline of feroxidase and antioxidant ceruloplasmin activity in the blood of rectal cancer patients may be the formation of NO combinations with this enzyme. The influence of radiation therapy on rectal cancer becomes apparent when the "free iron" combinations' levels and lipid peroxidation products are increased in the blood of these patients.

Keywords: Rectal cancer, Neoadjuvant Radiotherapy, Ceruloplasmin, Transferrin.

1. Introduction
Ceruloplasmin is a copper-containing protein. Its main functions include anti-oxidative action, the involvement in the copper transferring process and iron metabolism, the oxidation of biogenic amines. The significance of ceruloplasmin in the iron-exchange process is associated with the mobilization of iron from its deposits in organs followed by the inclusion of apotransferrin in trivalent state [1]. Ceruloplasmin contributes to this process by oxidizing Fe^{2+} ions to Fe^{3+} while possessing the feroxidase potency. The iron ions are transmitted to the tissues by transferrin. They are built into heme and iron capacious enzymes [1, 2, 3]. Ceruloplasmin is also required for withdrawal of copper from the tissues. It contains up to 95% of all copper blood plasma, and only 5-10% of it circulates in the bloodstream in the form of combinations with albumin and amino acids. In 1972 John Folkman revealed that the tumor cells primarily accumulate copper [4]. Cu^{2+} ion is a cofactor of different development agents. It is particularly an indispensable part of the tumor angiogenesis, thus ensuring its germination in the surrounding tissues, the weight growth and metastasizing. The use of drugs that specifically bind copper ions prevents tumor vascularization and metastasizing. The concentration of ceruloplasmin in blood varies depending on the general well-being. Ceruloplasmin is used for diagnosing and treating the patients in a number of pathological states, including malignant neoplasms. At the same time, there still remains an open question concerning the mechanisms regulating its activity and the protein concentration in the body, as well as the possibility of using it as a tumor marker and as a prognostic test [3].
The aim of the work was to study ceruloplasmin, transferrin levels, free iron combinations and to reveal ceruloplasmin-NO combinations in blood while treating rectal cancer patients and their influence on the antioxidant defense system status.

2 Materials and methods
2.1 Materials
The venous blood samples of 35 patients (18 men and 17 women, mean age 62 ± 1,8 years) have been studied. They were diagnosed with rectal adenocarcinoma of II – III stages ($T_{2-4}N_{0-2}M_0G_2$), when undergoing the treatment at Ivano-Frankivsk Regional Clinical Oncology Centre (Ukraine). In the first phase of treatment, patients received preoperative course of

distance gamma-ray therapy to the tumour region to a total focal dose of 39 Gy. A single focal dose constituted 3 Gy with 13 sessions during 2.5 weeks, 5 sessions per week. A radical surgical intervention was done 4-5 weeks after the last session of radiotherapy. The exclusion criteria of patients under the examination were the following: those who were over 80 years old; multiple primary character of tumour lesions; common grave condition of patients on admission to hospital due to the presence of accompanying systemic diseases in the stage of decompensation; intake of antioxidant vitamins by them a month before and during the research. It was conducted in compliance with the principles of biomedical research involving human subjects as indicated in the Declaration of Helsinki (1964) of the World Medical Association.

Blood taken for studies was sampled from rectal cancer patients prior to the treatment arrangements; after the 13th session of radiotherapy; in 4-5 weeks after finishing ray therapeutics (before the surgery). As a control marker, the blood indices of 15 healthy donors were examined. Trilon B was used as an anticoagulant. A test tube with 1 ml trilon B was filled with 5 ml of blood from the ulnar vein, where subsequently 0.5 ml were sampled and frozen in a special liquid nitrogen mold. Ceruloplasmin and transferrin levels, ceruloplasmin-nitric oxide (NO) combinations, free iron combinations were studied applying the electron paramagnetic resonance method (EPR) [5].

3. Results and Discussion

Modern EPR spectrometers make it possible to examine ceruloplasmin, transferrin, hemiglobin levels, ceruloplasmin-NO combinations, "free iron" systems in biological environments. Figure 1 displays a typical spectrum of EPR blood inherent in rectal cancer patients.

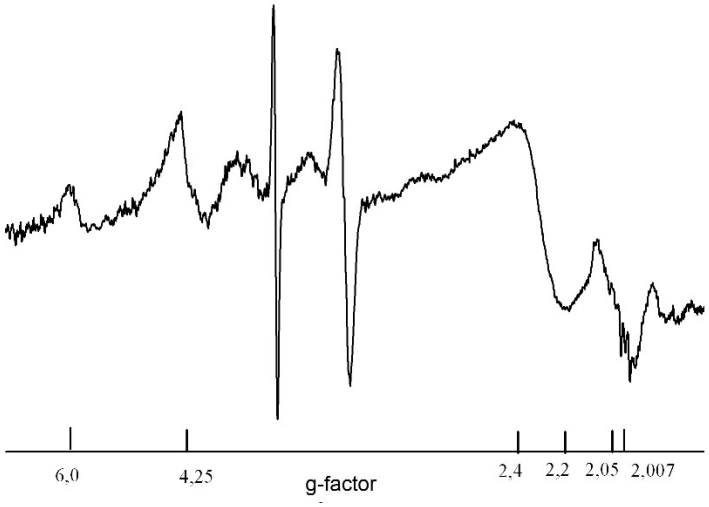

Fig 1: EPR blood spectrum of a rectal cancer patient.

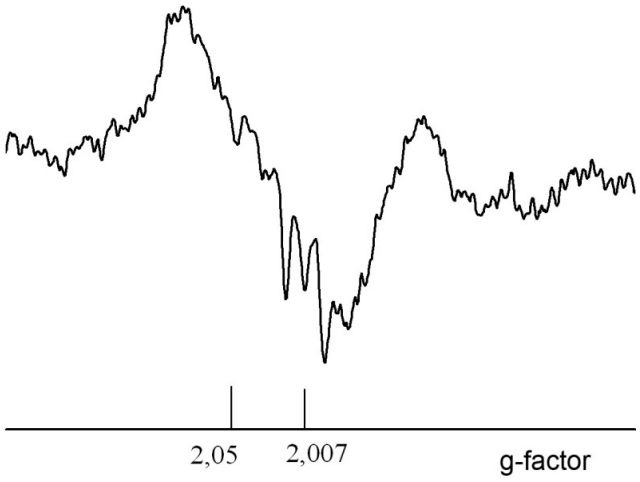

Fig 2: EPR ceruloplasmin-NO combination spectrum in the blood of rectal cancer patients.

The level of ceruloplasmin in blood is characterized by EPR signal with g = 2,05. In accordance with this spectrum we can determine ceruloplasmin concentration in the blood of rectal cancer patient. Ceruloplasmin has been registered as a complex with endogenous NO in the mentioned patient, the interaction of ceruloplasmin with NO and the combination formation hereat is indicated by a triplet EPR signal with g = 2,007. Methemoglobin and transferrin have also been revealed (g = 6,0 and g = 4,25 respectively). The combination level of "free iron" in blood is reflected by a broad EPR signal with g = 2,2 - 2,4.

The reducing of ceruloplasmin level in the blood of rectal

cancer patients may be due to the loss of ceruloplasmin peptide part of sialic acids residues. Another reason for reducing the circulating level of ceruloplasmin in blood may also be the loss of copper ions by a ceruloplasmin molecule. The appearance of the EPR signal at ceruloplasmin with g = 2,007 in rectal cancer patients indicates the formation of ceruloplasmin-NO combinations and the loss of physiological functions by ceruloplasmin (Fig.2.). This is the result of endogenous NO increase [6].

Copper type I is of great importance during the interaction process between ceruloplasmin and NO because it is the target for a nitric oxide molecule. Alongside with the formation of ceruloplasmin and NO combinations the reducing of the amount of copper in the active centres of ceruloplasmin can lead to suppression of the feroxidase and antioxidant metalloenzyme activity [7, 8].

Figure 3 displays the data about the activity of ceruloplasmin in donors' and rectal cancer patients' blood before the treatment; after the 13th session of radiotherapy; in 4-5 weeks after finishing radiotherapy (before the surgery). It has been discovered that the ceruloplasmin level in the blood of rectal cancer patients ranges within 0.36 to 0.49 limits averaging 0,41 ± 0,05 rel. units, which is about twice lower the level representative for the donors. The intensity of the EPR ceruloplasmin signal on average constitutes 0,78 ± 0,10 rel. units (Fig.3). After 13 sessions of neoadjuvant radiotherapy the ceruloplasmin concentration does not drastically change. Its amount varies in the range from 0.30 to 0.48 rel. units, on average 0,40 ± 0,04 rel. units, without any essential changes before starting the surgical treatment. Thus, the second phase of a combined therapy of rectal cancer patients, a traumatic and a long-term surgical intervention, is performed against the feroxidase and antioxidant ceruloplasmin activity failure mostly.

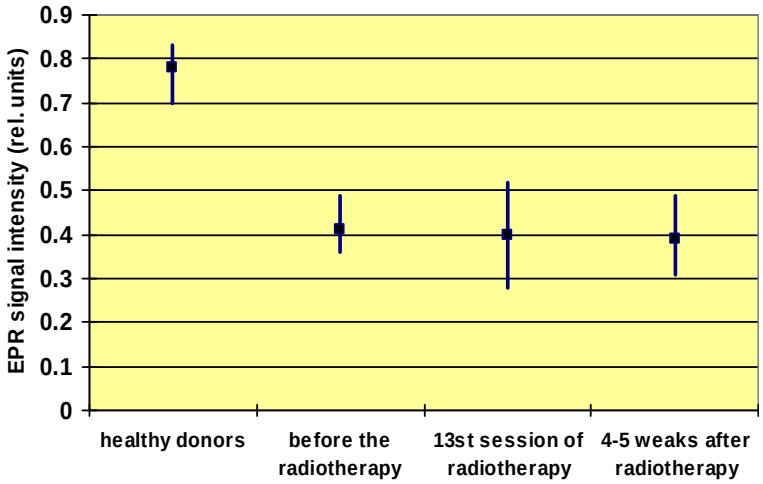

Fig 3: Ceruloplasmin activity in the donors' and rectal cancer patients' blood in different periods of radiotherapy.

During the study of transferrin concentration in the blood of patients with malignant tumors of rectum it has been revealed that transferrin decreases in 3.6 times compared to the rates of those from donors the EPR signal intensity of whose was at the level of 0,82 ± 0,11 rel. units. (Fig.4). After the 13th session of neoadjuvant radiotherapy we have observed the transferrin concentration's growth in 75% of patients in comparison with the average prior to the radiotherapy, whereas with the other patients this figure remained at the baseline. The transferrin level in the blood of rectal cancer patients before the operation was in the range of 0.28 to 0.5 rel. units, averaging 0,36 ± 0,05 relative units.

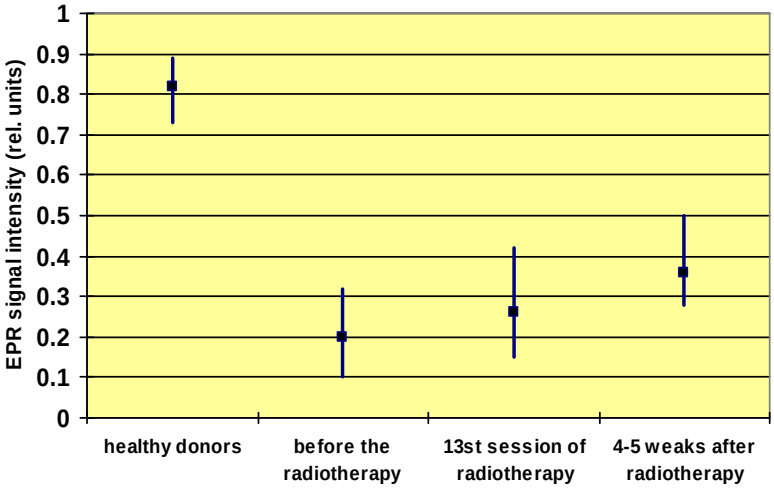

Fig 4: Transferrin level in the donors' and rectal cancer patients' blood in different periods of radiotherapy.

The main function of transferrin is its involvement in iron metabolism at the expense of its quality to bind and make Fe^{3+} ions easily. The data on concerted interactions between antioxidants ceruloplasmin and transferrin which have been obtained in recent years made it possible to identify the individual antioxidant system of blood serum – ceruloplasmin / transferring [1]. This coordinated action lies in that Fe^{2+} is assembled in an apotransferrin molecule provided it being oxidized to Fe^{3+} by feroxidase ceruloplasmin. Uncoupled iron ions are highly toxic in spite of the fact that they are vital for the functioning of biological systems. Transferrin+Fe^{3+} combination formation provides their converting into a soluble, non-toxic and readily available for the body's cells form. The role of transferrin as a transport protein is reduced to the transfer of iron from the place of its absorption to the liver and thence to the body's cells to take part in the synthesis of iron-containing enzymes. Fe^{3+} ions in particular are built in heme when synthesizing heme and non-heme proteins [5, 9]. Malignant cells are distinguished from benign ones when containing proteins of high concentration and activity involved in iron metabolism. This is primarily due to the fact that the tumor development requires a permanently high intracellular level of iron for the Fe-containing enzymes functioning that provide oxidative phenotype and cell proliferation [10].

The result of ceruloplasmin-transferrin system dysfunction and the degradation of heme-capacious and non-heme proteins in rectal cancer patients is the "free iron" combinations uptake. (Fig. 5). We have found that this index exceeded the normal level in patients' blood an order of magnitude more growing progradiently in the phase of treating the patients.

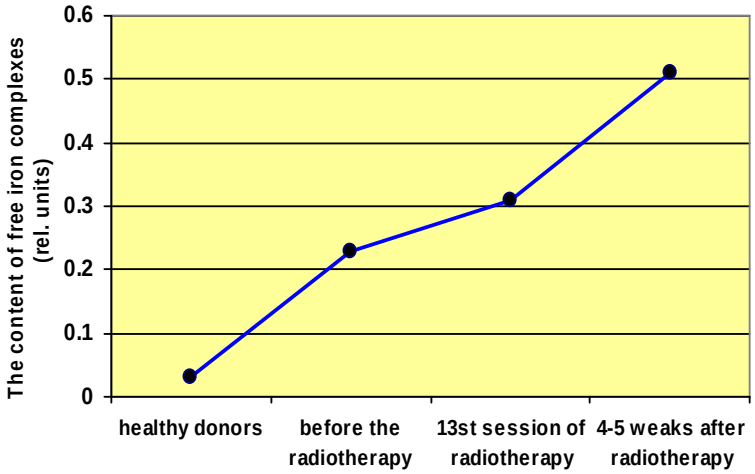

Fig 5: "Free iron" combinations' levels in the donors' and rectal cancer patients' blood in different periods of radiotherapy.

In the context of the above described changes of the ceruloplasmin-transferrin functional system and the growth of free iron concentration in the blood of patients the intensity of free radical processes have been significantly increasing. The representation of this process is the concentration of lipid peroxidation products (table). A significant increase of primary (diene conjugates) and secondary products (malonic dialdehyde) of lipids' oxidative modification is the evidence of free radical aggression against the physiological antioxidant defence system failure. Thus, we may assume that a "free iron" is the tool for strengthening the processes of oxidation in the body of rectal cancer patients.

Table: Level of lipid peroxidation indexes in the donors' and rectal cancer patients' blood in different periods of radiotherapy.

Index	Healthy donors	Index level before the radiotherapy	Index level after the 13-st session of radiotherapy	Index level 4-5 weaks after radiotherapy
Malonic dialdehyde (micromole/l)	3,12±0,27	4,23±0,49	7,11±0,33*	5,13±0,45*
Diene conjugates (standard units)	0,34±0,03	0,47±0,04*	0,88±0,07*	0,54±0,06*

* - P<0,05 compared with the index of healthy donors.

4. Conclusion

The results of the study show a reduction of ceruloplasmin and transferrin levels in the blood of rectal cancer patients prior to the treatment and a slight growth of transferrin when undergoing the course of radiotherapy. One of the reasons for the decline of feroxidase and antioxidant ceruloplasmin activity in the blood of rectal cancer patients may be the formation of NO combinations with this enzyme. The influence of radiation therapy on rectal cancer becomes apparent when the "free iron" combinations' levels and lipid peroxidation products are increased in the blood of these patients. Changes in ceruloplasmin, transferrin concentrations, the "free iron" combinations, ceruloplasmin-NO combinations are likely to be used when monitoring the disease behavior and the development of the therapeutic approaches to the correction of deviations in the redox state of the ceruloplasmin- transferrin system.

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